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Pharmacological Properties of Radiotracers That Measure P-glycoprotein Function and Density

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ABSTRACT

Energy-dependent transporters of the ATP-binding cassette (ABC) family regulate the movement of molecules across cellular membranes. Several of these transporters are expressed in the endothelial cells of brain microvessels (blood-brain barrier) to protect brain tissue from exposure to toxins in the blood. Three of the most common ABC transporters at the blood-brain barrier are P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance protein 1 (MRP1). Changes in P-gp function and density are hypothesized to play a role in neurological disorders, mediating drug-resistant epilepsy, drug effectiveness against HIV infection of the brain, and Alzheimer disease. Therefore, to measure P-gp function and density in vivo, substrates (which are transported by P-gp) and inhibitors (which bind to P-gp) have been radiolabeled for use in the nuclear imaging technique positron emission tomography (PET). For accurate quantification, radiotracers must be selective for P-gp and have high signal strength. The purpose of this thesis was to evaluate whether two radiotracers that are used to image P-gp function and density fulfill these properties.

The selectivity and signal strength of the P-gp substrate N-desmethyl-loperamide (dLop) and the P-gp inhibitor tariquidar were assessed using pharmacology assays in human cell lines and post-mortem mouse brains, and using PET imaging in transgenic mice and healthy humans. We found that the radiotracer [^{11}C]dLop is selective as a substrate for P-gp among the three major ABC transporters of the blood-brain barrier because accumulation of [^{3}H]dLop was lowest in cells expressing P-gp, and the uptake of [^{11}C]dLop was highest in brains of mice lacking P-gp. In addition to being selective, dLop is ionically trapped in acidic lysosomes; [^{3}H]dLop accumulation decreased by $\geq$ 50% in human cells pretreated with compounds that raise lysosomal pH. This irreversible trapping mechanism of [^{11}C]dLop amplifies the measured PET signal because radioactivity accumulates over time. However, the P-gp inhibitor tariquidar competes with dLop for lysosomal accumulation because it decreased the accumulation of [^{3}H]dLop by $\geq$ 50% in human cells and that of [^{11}C]dLop by $\geq$ 35-40% in lysosome-rich organs of P-gp knockout mice and healthy humans; competition was not observed in the brain. The lysosomal competition in the peripheral organs is problematic because tariquidar is used in combination with [^{11}C]dLop to measure P-gp function in vivo and suggests that these two compounds cannot be used together to measure P-gp function in the periphery.

We also found that tariquidar is not a specific inhibitor of P-gp; it is also a substrate and inhibitor of BCRP. At low concentrations, [^{3}H]tariquidar had highest accumulation in cells expressing P-gp and lowest accumulation in cells expressing BCRP, while at higher concentrations ($\geq$ 100 nM), tariquidar inhibited the function of both P-gp and BCRP. In addition to not being selective, [^{11}C]tariquidar has a low signal strength as a radiotracer because specific binding of [^{3}H]tariquidar to P-gp in post-mortem mouse brains was only 20-30% of the total signal.

In conclusion, the selectivity and high signal strength of the radiotracer [^{11}C]dLop allow it to selectively measure P-gp function at the blood-brain barrier and this radiotracer can be used to determine P-gp's role in neurological disorders. In contrast, the lack of selectivity and low signal strength of [^{11}C]tariquidar indicate that this inhibitor cannot measure P-gp density and that better inhibitor radiotracers are required.